



SEQUENCE LISTING

<110> Hoogenboom Hendricus R.J.M.
Hendrikx Maria P.G.

<120> MUCIN-1 Specific Binding Members and Methods of Use Thereof

<130> DYX-015.1 US

<140> 09/822,698

<141> 2001-03-30

<150> US 09/538,913

<151> 2000-03-30

<160> 112

<170> Microsoft Word

<210> 1

<211> 113

<212> PRT

<213> artificial sequence

<220>

<223> light chain variable region of the MUC1-specific
binding domain of PH1 Fab antibody

<400> 1

Glu Ile Val Leu Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Thr Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Ser Gly Ser His Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Val Ser Gly Thr Asp Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Gly
85 90 95

Leu Gln Ser Pro Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
100 105 110

Arg

<210> 2

<211> 339

<212> DNA

<213> artificial sequence

<220>

<223> nucleotide sequence coding for amino acid sequence of
SEQ ID NO:1

<400> 2

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gaaattgtgc tgactcagtc tccactctcc ctgcccgtca cccctggaga gccggcctcc      60
atctcctgca ggtctagtca gagcctcctg catagtaatg gatacaccta tttggattgg      120
tacctgcaga agccagggca gtctccacag ctctgatct attcgggttc tcatcgggcc      180
tccgggggtcc ctgacagggt cagtggcagt gtatcaggca cagattttac actgagaatc      240
agcagagtgg aggctgagga tgttggagtt tattactgca tgcagggtct acagagtcca      300
ttcactttcg gccctgggac caaagtggat atcaaacga                               339
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<210> 3

<211> 121

<212> PRT

<213> artificial sequence

<220>

<223> heavy chain variable region of the MUC1-specific
binding domain of the PH1 Fab antibody

<400> 3

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Gln Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10           15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Asn
          20           25           30

Ala Met Gly Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
          35           40           45

Ser Gly Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
          50           55           60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65           70           75           80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
          85           90           95

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Asp Tyr Trp Gly
          100          105          110

Gln Gly Thr Leu Val Thr Val Ser Ser
          115          120
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<210> 4
 <211> 363
 <212> DNA
 <213> artificial sequence

<220>
 <223> nucleotide sequence coding for amino acid sequence of
 SEQ ID NO:3

<400> 4

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caggtccagc tgggtgcagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc      60
tctctgtgcag cctctggatt cacgtttaga agtaacgcca tgggctgggt cgcagggt      120
ccaggaagg ggctggagtg ggtctcaggt attagtggta gtggtggcag cacatactac      180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat      240
ctgcaaatga acagcctgag agccgaggac acggccgtat attattgtgc gaaacatacc      300
ggggggggcg tttgggaccc cattgactac tggggccagg gaaccctggt caccgtctca      360
agc                                                                    363
  
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<210> 5
 <211> 381
 <212> PRT
 <213> artificial sequence

<220>
 <223> MUC1-specific immunocytokine bivPH1-IL-2

<400> 5

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Gln Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10           15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Asn
20           25           30
Ala Met Gly Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35           40           45
Ser Gly Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50           55           60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65           70           75           80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85           90           95
Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Asp Tyr Trp Gly
100          105          110
  
```

Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Ala Leu Glu Ile	115	120	125
Val Leu Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro	130	135	140
Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly	145	150	155
Tyr Thr Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln	165	170	175
Leu Leu Ile Tyr Ser Gly Ser His Arg Ala Ser Gly Val Pro Asp Arg	180	185	190
Phe Ser Gly Ser Val Ser Gly Thr Asp Phe Thr Leu Arg Ile Ser Arg	195	200	205
Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Gly Leu Gln	210	215	220
Ser Pro Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Arg Gly	225	230	235
Gly Gly Ser Gly Gly Gly Ala Leu Ala Pro Thr Ser Ser Ser Thr Lys	245	250	255
Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile	260	265	270
Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu	275	280	285
Thr Phe Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu	290	295	300
Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu	305	310	315
Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn	325	330	335
Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met	340	345	350
Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg	355	360	365
Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu Thr	370	375	380

<210> 6
 <211> 1143
 <212> DNA
 <213> artificial sequence

<220>
 <223> nucleotide sequence coding for amino acid sequence of
 SEQ ID NO:5

<400> 6

caggtccagc tgggtgcagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc	60
tcctgtgcag cctctggatt cacgtttaga agtaacgcca tgggctgggt ccgccagget	120
ccaggggaagg ggctggagtg ggtctcaggt attagtggta gtggtggcag cacatactac	180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat	240
ctgcaaatga acagcctgag agccgaggac acggccgtat attattgtgc gaaacatacc	300
ggggggggcg tttgggaccc cattgactac tggggccagg gaaccctgggt caccgtctca	360
agcggaggcg gtgcacttga aattgtgctg actcagtctc cactctccct gcccgtcacc	420
cctggagagc cggcctccat ctctgcagg tctagtcaga gcctcctgca tagtaatgga	480
tacacctatt tggattggta cctgcagaag ccagggcagt ctccacagct cctgatctat	540
tcgggttctc atcgggcctc cggggtcctt gacaggttca gtggcagtggt atcaggcaca	600
gattttacac tgagaatcag cagagtggag gctgaggatg ttggagttta ttactgcatg	660
cagggctctac agagtccatt cactttcggc cctgggacca aagtggatat caaacgaggg	720
ggtggatcag gcggcggggc ctagcacct acttcaagtt ctacaaagaa aacacagcta	780
caactggagc atttactgct ggatttacag atgattttga atggaattaa taattacaag	840
aatcccaaac tcaccaggat gtcacattt aagttttaca tgcccaagaa ggccacagaa	900
ctgaaacatc ttcagtgtct agaagaagaa ctcaaacctc tggaggaagt gctaaattta	960
gctcaaagca aaaactttca cttaagaccc agggacttaa tcagcaatat caacgtaata	1020
gttctggaac taaagggatc tgaaacaaca ttcattgtgtg aatatgctga tgagacagca	1080
accattgtag aatttctgaa cagatggatt accttttgtc aaagcatcat ctcaacactg	1140
act	1143

<210> 7
 <211> 20
 <212> PRT
 <213> artificial sequence

<220>
 <223> peptide of MUC1 protein

<400> 7

Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro Gly
 1 5 10 15
 Ser Thr Ala Pro
 20

<210> 8
 <211> 20
 <212> PRT
 <213> artificial sequence

<220>
 <223> peptide of MUC1 protein

<400> 8

Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala Pro
 1 5 10 15

Pro Ala His Gly
 20

<210> 9
 <211> 24
 <212> DNA
 <213> artificial sequence

<220>
 <223> CH1FOR primer

<400> 9

gtccttgacc aggcagccca gggc

24

<210> 10
 <211> 23
 <212> DNA
 <213> artificial sequence

<220>
 <223> pUC-reverse primer

<400> 10

agcggataac aatttcacac agg

23

<210> 11
 <211> 44
 <212> DNA
 <213> artificial sequence

<220>
 <223> VL backward primer

<400> 11

accgcctcca ccagtgcaact tgaaattgtg ctgactcagt ctcc 44

<210> 12

<211> 51

<212> DNA

<213> artificial sequence

<220>

<223> VL forward primer

<400> 12

accgcctcca ccgggcgcgc cttattaaca ctctcccctg ttgaagctct t 51

<210> 13

<211> 61

<212> DNA

<213> artificial sequence

<220>

<223> VL backward primer for light chain variable region
of the PH1 Fab antibody with additional linker and
restriction sites

<400> 13

gccgatcgct ctggtcaccg tctcaagcgg aggcggtgca cttgaaattg tgctgactca 60

g 61

<210> 14

<211> 50

<212> DNA

<213> artificial sequence

<220>

<223> VL forward primer for light chain variable region
of PH1 Fab antibody with additional linker and
restriction sites

<400> 14

gtctcgcgag cggccgccga ttggatatcc actttggtcc cagggccgaa 50

<210> 15

<211> 27

<212> DNA

<213> artificial sequence

<220>

<223> nucleotide sequence coding for a

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          nine amino acid linker

<400> 15

gggggtggat caggcggcgg ggccta                27

<210> 16
<211> 69
<212> DNA
<213> artificial sequence

<220>
<223> PH1-IL-2 backward primer

<400> 16

accaaagtgg atatcaaacg agggggtgga tcaggcggcg gggccctagc acctacttca        60

agttctaca                                     69

<210> 17
<211> 49
<212> DNA
<213> artificial sequence

<220>
<223> PH1-IL-2 forward primer

<400> 17

gtcccgcgtg cggccgcagt cagtgttgag atgatgcttt gacaaaagg                49

<210> 18
<211> 98
<212> PRT
<213> artificial sequence

<220>
<223> heavy chain variable region from a DP47 germ line

<400> 18

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
          20          25          30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
          35          40          45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
          50          55          60

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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys

<210> 19
<211> 100
<212> PRT
<213> artificial sequence

<220>
<223> light chain variable region from a DPK 15 germ line

<400> 19

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
85 90 95

Leu Gln Thr Pro
100

<210> 20
<211> 14
<212> PRT
<213> artificial sequence

<220>
<223> myc tag peptide

<400> 20

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Gly Ala Ala
1 5 10

<210> 21
<211> 42

<212> DNA
<213> artificial sequence

<220>
<223> nucleotide sequence coding for amino acid sequence
of SEQ ID NO:20

<400> 21

gaacaaaaac tcattctcaga agaggatctg aatggggccg ca

42

<210> 22
<211> 6
<212> PRT
<213> artificial sequence

<220>
<223> hexahistidine peptide

<400> 22

His His His His His His
1 5

<210> 23
<211> 18
<212> DNA
<213> artificial sequence

<220>
<223> nucleotide sequence coding for amino acid sequence
of SEQ ID NO:22

<400> 23

catcaccatc atcaccat

18

<210> 24
<211> 220
<212> PRT
<213> artificial sequence

<220>
<223> immunoglobulin kappa light chain of MUC1-specific PH1-IgG1

<400> 24

Glu Ile Val Leu Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Thr Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser

35					40					45					
Pro	Gln	Leu	Leu	Ile	Tyr	Ser	Gly	Ser	His	Arg	Ala	Ser	Gly	Val	Pro
50						55				60					
Asp	Arg	Phe	Ser	Gly	Ser	Val	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Arg	Ile
65				70						75				80	
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Met	Gln	Gly
				85				90						95	
Leu	Gln	Ser	Pro	Phe	Thr	Phe	Gly	Pro	Gly	Thr	Lys	Val	Asp	Ile	Lys
		100						105				110			
Arg	Gly	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp
		115				120						125			
Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn
130						135				140					
Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu
145				150						155				160	
Gln	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp
				165				170						175	
Ser	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr
		180						185				190			
Glu	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser
195						200						205			
Ser	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys				
210						215				220					

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<210> 25
<211> 663
<212> DNA
<213> artificial sequence
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<223> nucleotide sequence coding for amino acid sequence of
SEQ ID NO:24

gaaattgtgc	tgactcagtc	tccactctcc	ctgcccgtca	cccctggaga	gccggcctcc	60
atctcctgca	ggtctagtca	gagcctcctg	catagtaatg	gatacaccta	tttggattgg	120
tacctgcaga	agccagggca	gtctccacag	ctcctgatct	attcggggttc	tcatcggggcc	180
tccgggggtcc	ctgacagggt	cagtggcagt	gtatcaggca	cagattttac	actgagaatc	240
aqcagagtgg	aggctgagga	tgttggagtt	tattactgca	tgcagggtct	acagagtcca	300

ttcacttttcg gccctgggac caaagtggat atcaaacgag gaactgtggc tgcaccatct 360
gtcttcatct tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgccctc 480
caatcgggta actcccagga gagtgtcaca gagcaggaca gcaaggacag cacctacagc 540
ctcagcagca ccctgacgct gagcaaagca gactacgaga aacacaaagt ctacgcctgc 600
gaagtcaccc atcagggcct gagttcaccg gtgacaaaga gcttcaacag gggagagtgt 660
tag 663

<210> 26
<211> 451
<212> PRT
<213> artificial sequence

<220>
<223> immunoglobulin heavy chain of MUC1-specific PH1-IgG1

<400> 26

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Asn
20 25 30

Ala Met Gly Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175

Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	
			180					185					190			
Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	
			195				200					205				
Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	
	210					215					220					
Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	
225					230					235					240	
Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	
				245					250						255	
Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	
			260					265					270			
Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	
		275					280					285				
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	
	290					295					300					
Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	
305					310					315					320	
Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	
				325					330					335		
Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	
			340					345					350			
Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	
		355					360					365				
Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	
	370					375					380					
Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	
385					390					395					400	
Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	
				405					410					415		
Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	
			420					425					430			
His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	
		435					440					445				
Pro	Gly	Lys														
		450														

<210> 27

[illegible][illegible][illegible][illegible]

<210> 28
 <211> 14
 <212> PRT
 <213> artificial sequence

 <220>
 <221> variant
 <222> (1)..(14)
 <223> amino acid positions designated Xaa may be varied
 to form alternative regions of a MUC1-specific
 binding member as explained in the disclosure

 <400> 28

Xaa Xaa His Thr Gly Xaa Gly Val Trp Xaa Pro Xaa Xaa Xaa
 1 5 10

<210> 29
 <211> 14
 <212> PRT
 <213> artificial sequence

 <220>
 <223> region of a MUC1-specific binding member

 <400> 29
 Ala Lys His Thr Gly Arg Gly Val Trp Asp Pro Ile Gly Tyr
 1 5 10

<210> 30
 <211> 14
 <212> PRT
 <213> artificial sequence

 <220>
 <223> region of a MUC1-specific binding member

 <400> 30
 Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Lys His
 1 5 10

<210> 31
 <211> 14
 <212> PRT
 <213> artificial sequence

 <220>
 <223> region of a MUC1-specific binding member

 <400> 31
 Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Gly Tyr

1

5

10

<210> 32
 <211> 14
 <212> PRT
 <213> artificial sequence

 <220>
 <223> amino acid sequence in a MUC1-specific binding member

 <400> 32

Ala Ile His Thr Gly Gly Gly Val Trp Asp Pro Ile Lys Tyr
 1 5 10

<210> 33
 <211> 33
 <212> DNA
 <213> artificial sequence

 <220>
 <221> misc_feature
 <222> (1)..(33)
 <223> n may be varied according to the disclosure
 to form mutagenic primer sequences

<400> 33
 ggattcacgt ttagannnaa cgccatgggc tgg

33

<210> 34
 <211> 39
 <212> DNA
 <213> artificial sequence

<220>
 <221> misc_feature
 <222> (1)..(39)
 <223> n may be varied according to the disclosure
 to form mutagenic primer sequences

<400> 34

cacggagtct gcgtannntg tnnngccacc actaccact

39

<210> 35
 <211> 90
 <212> DNA
 <213> artificial sequence

<220>
 <221> misc_feature
 <222> (1)..(90)

[illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible]

<211> 16
 <212> PRT
 <213> artificial sequence

<220>
 <223> mutant CDR3 region of an antibody heavy chain variable region

<400> 43

Ala	Lys	Ser	Ser	Thr	Thr	Thr	Val	Trp	Asp	Pro	Ile	Asp	Tyr	Trp	Gly
1				5				10						15	

<210> 44
 <211> 48
 <212> DNA
 <213> artificial sequence

<220>
 <223> nucleotide sequence coding for a mutant CDR3 region

<400> 44

gcgaaatcta gtactacgac ggtttgggac cccattgact actggggc 48

<210> 45
 <211> 16
 <212> PRT
 <213> artificial sequence

<220>
 <221> variant
 <222> (1)..(16)
 <223> Xaa, designated as "&" in the disclosure, indicates end of amino acid sequence because mutation in nucleotide sequence forms a translational stop codon

<400> 45

Ala	Lys	Xaa	Pro	Met	Ala	Asn	Val	Trp	Asp	Pro	Ile	Asp	Tyr	Trp	Gly
1				5				10						15	

<210> 46
 <211> 48
 <212> DNA
 <213> artificial sequence

<220>
 <223> nucleotide sequence coding for a mutant CDR3 region

<400> 46

gcgaaatagc ctatggcgaa tgtttgggac cccattgact actggggc 48

<210> 47
 <211> 16
 <212> PRT
 <213> artificial sequence

<220>
 <221> variant
 <222> (1)..(16)
 <223> Xaa, designated as "&" in the disclosure, indicates end
 of amino acid sequence because mutation in nucleotide
 sequence forms a translational stop codon

<400> 47

Ala	Lys	Xaa	His	Thr	Lys	Thr	Val	Trp	Asp	Pro	Ile	Asp	Tyr	Trp	Gly
1				5				10						15	

<210> 48
 <211> 48
 <212> DNA
 <213> artificial sequence

<220>
 <223> nucleotide sequence coding for a mutant CDR3 region

<400> 48

gcgaaatagc atacgaagac ggtttgggac cccattgact actggggc 48

<210> 49
 <211> 16
 <212> PRT
 <213> artificial sequence

<220>
 <223> mutant CDR3 region of an antibody heavy chain variable region

<400> 49

Ala	Lys	Ile	Thr	Val	Ser	Arg	Val	Trp	Asp	Pro	Ile	Asp	Tyr	Trp	Gly
1				5				10						15	

<210> 50
 <211> 48
 <212> DNA
 <213> artificial sequence

<220>
 <223> nucleotide sequence coding for a mutant CDR3 region

<400> 50

gcgaaaatta ctgtagtcg tgtttgggac cccattgact actggggc 48

109020"3593360

<210> 51
<211> 16
<212> PRT
<213> artificial sequence

<220>
<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 51

Ala Lys Arg Tyr Leu Tyr Asp Val Trp Asp Pro Ile Asp Tyr Trp Gly
1 5 10 15

<210> 52
<211> 48
<212> DNA
<213> artificial sequence

<220>
<223> nucleotide sequence coding for a mutant CDR3 region

<400> 52

gcgaaacgtt atctgtatga tgtttgggac cccattgact actggggc 48

<210> 53
<211> 16
<212> PRT
<213> artificial sequence

<220>
<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 53

Ala Lys His Thr Gly Gly Gly Thr Leu Gln Arg Leu Asp Tyr Trp Gly
1 5 10 15

<210> 54
<211> 48
<212> DNA
<213> artificial sequence

<220>
<223> nucleotide sequence coding for a mutant CDR3 region

<400> 54

gcgaaacata cggggggggg cactttgcag cggctggact actggggc 48

<210> 55
<211> 16

105020 35922860

<212> PRT
<213> artificial sequence

<220>
<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 55

Ala Lys His Thr Gly Gly Gly Thr Gln Thr Pro Cys Asp Tyr Trp Gly
1 5 10 15

<210> 56
<211> 48
<212> DNA
<213> artificial sequence

<220>
<223> nucleotide sequence coding for a mutant CDR3 region

<400> 56

gcgaaacata ccggggggggg cactcagact ccgtgtgact actgggggc 48

<210> 57
<211> 16
<212> PRT
<213> artificial sequence

<220>
<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 57

Ala Lys His Thr Gly Gly Gly Arg Arg Ile Cys His Asp Tyr Trp Gly
1 5 10 15

<210> 58
<211> 48
<212> DNA
<213> artificial sequence

<220>
<223> nucleotide sequence coding for a mutant CDR3 region

<400> 58

gcgaaacata ccggggggggg ccgtcgtatt tgatcatgact actgggggc 48

<210> 59
<211> 16
<212> PRT
<213> artificial sequence

<220>
 <221> variant
 <222> (1)..(16)
 <223> Xaa, designated as "&" in the disclosure, indicates end
 of amino acid sequence because mutation in nucleotide
 sequence forms a translational stop codon

<400> 59

Ala	Lys	His	Thr	Gly	Gly	Gly	Xaa	Arg	Xaa	Xaa	Arg	Asp	Tyr	Trp	Gly
1				5				10						15	

<210> 60
 <211> 48
 <212> DNA
 <213> artificial sequence

<220>
 <223> nucleotide sequence coding for a mutant CDR3 region

<400> 60

gcgaaacata ccgsgggggggg ctagcggtag tagcgggact actgggggc

48

<210> 61
 <211> 16
 <212> PRT
 <213> artificial sequence

<220>
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<400> 61

Ala	Lys	His	Thr	Gly	Gly	Gly	Gln	Lys	Leu	Gln	Leu	Asp	Tyr	Trp	Gly
1				5				10						15	

<210> 62
 <211> 48
 <212> DNA
 <213> artificial sequence

<220>
 <223> nucleotide sequence coding for a mutant CDR3 region

<400> 62

gcgaaacata ccgsgggggggg ccagaagctg cagctggact actgggggc

48

<210> 63
 <211> 16
 <212> PRT
 <213> artificial sequence

<220>
 <221> variant
 <222> (1)..(16)
 <223> Xaa, when designated as "&" in the disclosure, indicates end of amino acid sequence because mutation in nucleotide sequence forms a translational stop codon; or when designated as "s" in the disclosure, Xaa is serine

<400> 63

Ala	Xaa	His	Thr	Gly	Gly	Arg	Gly	Trp	Asp	Pro	Ile	Asp	Tyr	Trp	Gly
1				5				10						15	

<210> 64
 <211> 48
 <212> DNA
 <213> artificial sequence

<220>
 <223> nucleotide sequence coding for a mutant CDR3 region

<400> 64

gcgtsacata cgggggggcg cggttgggac cccattgact actggggc

48

<210> 65
 <211> 16
 <212> PRT
 <213> artificial sequence

<220>
 <223> mutant CDR3 region of an antibody heavy chain variable region

<400> 65

Ala	Asn	Gln	Thr	Gly	Gly	Gly	Val	Trp	Asp	Pro	Ile	Asp	Tyr	Trp	Gly
1				5				10						15	

<210> 66
 <211> 48
 <212> DNA
 <213> artificial sequence

<220>
 <223> nucleotide sequence coding for a mutant CDR3 region

<400> 66

gcgaaccaga ctgggggggg cgtttgggac cccattgact actggggc

48

<210> 67
 <211> 16

Sequence 4869-4870

<212> PRT
<213> artificial sequence

<220>
<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 67

Ala Arg His Thr Gly Gly Gly Val Trp Asp Pro Ile Tyr Tyr Trp Gly
1 5 10 15

<210> 68
<211> 48
<212> DNA
<213> artificial sequence

<220>
<223> nucleotide sequence coding for a mutant CDR3 region

<400> 68

gcgagacata ccggtggggg cgtktgggat cccatatact actggggc 48

<210> 69
<211> 16
<212> PRT
<213> artificial sequence

<220>
<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 69

Ala Lys Pro Thr Gly Gly Gly Ala Trp Asp Pro Ile Asp Tyr Trp Gly
1 5 10 15

<210> 70
<211> 48
<212> DNA
<213> artificial sequence

<220>
<223> nucleotide sequence coding for a mutant CDR3 region

<400> 70

gcgaaaccta ccgggggggg cgcttgggac cccattgact actggggc 48

<210> 71
<211> 16
<212> PRT
<213> artificial sequence

<220>
<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 71

Ala Lys His Thr Gly Val Gly Val Trp His Pro Ile Tyr Tyr Trp Gly
1 5 10 15

<210> 72
<211> 48
<212> DNA
<213> artificial sequence

<220>
<223> nucleotide sequence coding for a mutant CDR3 region

<400> 72

gcgaaacata ccgggggtggg cgtttggcac cccatctact actggggc

48

<210> 73
<211> 14
<212> PRT
<213> artificial sequence

<220>
<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 73

Ala Lys His Thr Gly Val Gly Val Trp Asp Pro Ile Lys Tyr
1 5 10

<210> 74
<211> 14
<212> PRT
<213> artificial sequence

<220>
<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 74

Ala Lys His Thr Gly Glu Gly Val Trp Asp Pro Ile Lys Tyr
1 5 10

<210> 75
<211> 14
<212> PRT
<213> artificial sequence

<220>
<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 75

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Asp Lys
1 5 10

<210> 76

<211> 14

<212> PRT

<213> artificial sequence

<220>

<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 76

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Gly Tyr
1 5 10

<210> 77

<211> 14

<212> PRT

<213> artificial sequence

<220>

<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 77

Ala Arg His Thr Gly Gly Gly Val Trp Asp Pro Ile Gly Tyr
1 5 10

<210> 78

<211> 14

<212> PRT

<213> artificial sequence

<220>

<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 78

Ser Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Gly Tyr
1 5 10

<210> 79

<211> 14

<212> PRT

<213> artificial sequence

<220>

<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 79

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Gly His
1 5 10

<210> 80

<211> 14

<212> PRT

<213> artificial sequence

<220>

<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 80

Ala Lys His Thr Gly Gly Gly Val Trp Asn Pro Ile Gly His
1 5 10

<210> 81

<211> 14

<212> PRT

<213> artificial sequence

<220>

<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 81

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Leu Gly Tyr
1 5 10

<210> 82

<211> 14

<212> PRT

<213> artificial sequence

<220>

<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 82

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Leu Asp Asn
1 5 10

<210> 83

<211> 14

<212> PRT

<213> artificial sequence

<220>

<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 83

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Asn Tyr
1 5 10

<210> 84
<211> 14
<212> PRT
<213> artificial sequence

<220>
<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 84

Ala Arg His Thr Gly Gly Gly Val Trp Asp Pro Ile Asn Tyr
1 5 10

<210> 85
<211> 14
<212> PRT
<213> artificial sequence

<220>
<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 85

Ala Lys His Thr Gly Ser Gly Val Trp Asp Pro Ile Asn Tyr
1 5 10

<210> 86
<211> 14
<212> PRT
<213> artificial sequence

<220>
<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 86

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Asn Asp
1 5 10

<210> 87
<211> 14
<212> PRT
<213> artificial sequence

<220>
<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 87

Ala Lys His Thr Gly Val Gly Val Trp Asp Pro Met Asn Tyr
 1 5 10

<210> 88
 <211> 14
 <212> PRT
 <213> artificial sequence

<220>
 <223> mutant CDR3 region of an antibody heavy chain variable region

<400> 88

Thr Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Asn Tyr
 1 5 10

<210> 89
 <211> 14
 <212> PRT
 <213> artificial sequence

<220>
 <223> mutant CDR3 region of an antibody heavy chain variable region

<400> 89

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Ala Tyr
 1 5 10

<210> 90
 <211> 14
 <212> PRT
 <213> artificial sequence

<220>
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<400> 90

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Ala Asn
 1 5 10

<210> 91
 <211> 14
 <212> PRT
 <213> artificial sequence

<220>
 <223> mutant CDR3 region of an antibody heavy chain variable region

<400> 91

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Phe Ala Tyr

1 5 10

<210> 92
 <211> 14
 <212> PRT
 <213> artificial sequence

<220>
 <223> mutant CDR3 region of an antibody heavy chain variable region
 <400> 92

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Met Ala Ser
 1 5 10

<210> 93
 <211> 14
 <212> PRT
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<220>
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 <400> 93

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Met Asp Tyr
 1 5 10

<210> 94
 <211> 14
 <212> PRT
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<220>
 <223> mutant CDR3 region of an antibody heavy chain variable region
 <400> 94

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile His Tyr
 1 5 10

<210> 95
 <211> 14
 <212> PRT
 <213> artificial sequence

<220>
 <223> mutant CDR3 region of an antibody heavy chain variable region
 <400> 95

Ala Ile His Thr Gly Ala Gly Val Trp Asp Pro Ile Arg Tyr
 1 5 10

<210> 96
 <211> 14
 <212> PRT
 <213> artificial sequence

<220>
 <223> mutant CDR3 region of an antibody heavy chain variable region
 <400> 96

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Ser Ser
 1 5 10

<210> 97
 <211> 14
 <212> PRT
 <213> artificial sequence

<220>
 <223> mutant CDR3 region of an antibody heavy chain variable region
 <400> 97

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Asp Asp
 1 5 10

<210> 98
 <211> 14
 <212> PRT
 <213> artificial sequence

<220>
 <223> mutant CDR3 region of an antibody heavy chain variable region
 <400> 98

Val Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Val Tyr
 1 5 10

<210> 99
 <211> 14
 <212> PRT
 <213> artificial sequence

<220>
 <223> mutant CDR3 region of an antibody heavy chain variable region
 <400> 99

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Val Asp Tyr
 1 5 10

<210> 100
 <211> 14
 <212> PRT
 <213> artificial sequence

 <220>
 <223> mutant CDR3 region of an antibody heavy chain variable region

 <400> 100

 Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Val Pro
 1 5 10

<210> 101
 <211> 14
 <212> PRT
 <213> artificial sequence

 <220>
 <223> mutant CDR3 region of an antibody heavy chain variable region

 <400> 101

 Val Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Ala Tyr
 1 5 10

<210> 102
 <211> 14
 <212> PRT
 <213> artificial sequence

 <220>
 <223> mutant CDR3 region of an antibody heavy chain variable region

 <400> 102

 Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile His Asn
 1 5 10

<210> 103
 <211> 14
 <212> PRT
 <213> artificial sequence

 <220>
 <223> mutant CDR3 region of an antibody heavy chain variable region

 <400> 103

 Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Met His Tyr
 1 5 10

<210> 104
 <211> 14

<212> PRT
 <213> artificial sequence

 <220>
 <223> mutant CDR3 region of an antibody heavy chain variable region

 <400> 104

Ala Lys His Thr Gly Gly Gly Val Trp Asn Pro Ile Asp Tyr
 1 5 10

<210> 105
 <211> 14
 <212> PRT
 <213> artificial sequence

 <220>
 <223> mutant CDR3 region of an antibody heavy chain variable region

 <400> 105

Val Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Asp Tyr
 1 5 10

<210> 106
 <211> 14
 <212> PRT
 <213> artificial sequence

 <220>
 <223> mutant CDR3 region of an antibody heavy chain variable region

 <400> 106

Ala Lys His Thr Gly Ala Gly Val Trp Asp Pro Ile Asp Tyr
 1 5 10

<210> 107
 <211> 14
 <212> PRT
 <213> artificial sequence

 <220>
 <223> mutant CDR3 region of an antibody heavy chain variable region

 <400> 107

Ala Gln His Thr Gly Gly Gly Val Trp Asp Pro Ile Gly Tyr
 1 5 10

<210> 108
 <211> 14
 <212> PRT

<213> artificial sequence

<220>

<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 108

Ala Lys His Thr Gly Arg Gly Val Trp Asp Pro Ile Asp Tyr
1 5 10

<210> 109

<211> 14

<212> PRT

<213> artificial sequence

<220>

<223> mutant CDR3 region of an antibody heavy chain variable region

<400> 109

Ala Lys His Thr Gly Gly Gly Val Trp Asp Pro Ile Tyr Tyr
1 5 10

<210> 110

<211> 66

<212> DNA

<213> artificial sequence

<220>

<223> VH1C Back eukaryotic primer

<400> 110

ggactagtcc tggagtgcgc gcaactcccag gtccagctgg tgcagtctgg gggaggcttg 60
gtacag 66

<210> 111

<211> 73

<212> DNA

<213> artificial sequence

<220>

<223> VKexpress-MUC-for primer

<400> 111

gcgctcgcat ttgcctgtta attaagttag atctattcta ctcacgtttg atatccactt 60
tggtcccagg gcc 73

<210> 112

<211> 35

<212> DNA
<213> artificial sequence

<220>
<223> MUC1-VL-Back-APA primer

<400> 112

ccagtgcact ccgaaattgt gctgactcag tctcc

35

09/09/2019 10:06:04